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55-Base Pair Deletion in Certain Patients With Gaucher Disease Complicates Screening for Common Gaucher Alleles

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Mutations in the glucocerebrosidase gene which result in Gaucher disease can originate from the highly homologous glucocerebrosidase pseudogene. A 55-bp deletion in exon 9, which corresponds to a 55-bp segment absent from the pseudogene, has been identified in patients with Gaucher disease. We have developed a simple polymerase chain reaction (PCR)-based method to detect this 55-bp deletion, and have found this mutation in 3 of 75 DNA samples (4%) collected from patients with Gaucher disease. Commonly used PCR-based screening methods for specific Gaucher mutations frequently make use of primers either within or surrounding the 55-bp gap to selectively distinguish the glucocerebrosidase gene from the pseudogene. However, if the 55-bp deletion in exon 9 occurs, primers will either fail to produce an amplification product or will produce a shortened product which will be falsely attributed to the pseudogene. This could lead to inaccurate genotyping and genetic counseling for some Gaucher patients and their families. We therefore recommend that laboratories using PCR-based screening techniques involving primers in this region initially determine whether this 55-bp sequence is present. © 1996 Wiley-Liss, Inc.

KEY WORDS: Gaucher disease, glucocerebrosidase, pseudogene, deletion, genotyping

INTRODUCTION

Gaucher disease, the inherited deficiency of the enzyme glucocerebrosidase (EC 3.2.1.45), is characterized by both phenotypic and genotypic heterogeneity [Beutler and Grabowski, 1995; Sidransky and Ginns, 1993; Martin et al., 1989]. While there are several relatively common mutant alleles encountered among patients with Gaucher disease, more than 50 different mutations have been described and the number of mutations identified is continually expanding [Beutler et al., 1993, 1995; Beutler and Grabowski, 1995; Horowitz and Zimran, 1994; Uchiyama et al., 1994]. Recently a 55-base pair deletion in exon 9 of the glucocerebrosidase gene (Fig. 1) has been identified as a mutation in patients with Gaucher disease [Beutler et al., 1995; Walley and Harris, 1993]. This 55-base pair segment is particularly interesting because it is also absent in the glucocerebrosidase pseudogene, a highly homologous sequence located downstream to the glucocerebrosidase gene [Reiner et al., 1988]. There is a number of mutant alleles in patients with Gaucher disease which arise from recombination or fusion events between the glucocerebrosidase gene and pseudogene [Beutler and Grabowski, 1995; Horowitz and Zimran, 1994].

Many laboratories have used this 55-bp sequence as a means to selectively distinguish the glucocerebrosidase gene sequence from the pseudogene sequence by designing polymerase chain reaction (PCR) primers in or around the 55-bp pseudogene gap [Theophilus et al., 1989; Choy et al., 1991; Sidransky et al., 1992; Eyal et al., 1990; Zimran et al., 1990; Beutler et al., 1990; Laubscher et al., 1994]. However, when the 55 base pairs are deleted from a glucocerebrosidase allele, the use of these primers may lead to incorrect or incomplete genotyping.

In this report we describe additional patients with this 55-bp deletion which were missed by conventional mutation screening techniques. We present a simple PCR assay to establish whether the 55-base pair segment is deleted in DNA samples from patients with Gaucher disease, and discuss the implications of this deletion when interpreting genotype results.

MATERIALS AND METHODS

Genomic DNA was isolated from cultured cell lines from 75 unrelated patients with Gaucher disease [Sambrook et al., 1989]. The DNA was screened for the

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Fig. 1. Schematic representation of exons 9 and 10 of the glucocerebrosidase gene. The 55-bp sequence absent in the pseudogene is indicated. A and B denote the location of the two primers used to amplify a 484 bp fragment. Primers 1, 2, and 3 are those used in ARMS method to detect the N370S mutation (asterisk).

presence of 5 common Gaucher mutations as previously described [Sidransky et al., 1994; Mistry et al., 1992]. Mutation N370S was initially detected using an amplification refractory mutation system (ARMS) [Mistry et al., 1992] (Fig. 1, primers 1, 2, and 3). In order to detect the 55-bp deletion, a 484-bp genomic fragment spanning exon 9 and parts of introns 8 and 9 was selectively amplified by PCR from the glucocerebrosidase gene but not the pseudogene (Fig. 1, primers A and B). Positive and negative controls were included using plasmids containing only the glucocerebrosidase gene and pseudogene, respectively. The sequence of primer A from intron 8 was 5′-AACCATGATTCCCTATCTTC-3′ with the underlined nucleotides denoting where the glucocerebrosidase gene and pseudogene sequence differ. The sequence of the reverse primer B from intron 9 was 5′-GCTCCCTCGTGGTGTAGAGT-3′. PCR amplification reactions were prepared in 50 μl volumes, containing 200–500 ng genomic DNA, 1.0 μM of each primer, 5 μl 10X standard PCR buffer with 25 mM $Mgcl_2$, 2.0 μM of each dNTP and 2.5 U of Taq DNA polymerase. PCR was performed with a 6-min denaturation at 94°C, followed by 30 cycles of 1 min denaturation at 94°C, 2 min annealing at 56°C, 2 min extension of 72°C and a final extension of 10 min at 72°C. The PCR product was then electrophoresed on 2% agarose gel. When 2 bands were detected (Fig. 2), each amplified band was individually excised from the gel and purified using GeneClean II (Bio 101, LaJolla, CA). Direct PCR and cycle sequencing were performed on each sample according to the Taq DiDeoxy™ terminator kit protocol (Perkin Elmer, Foster City, CA).

RESULTS

Detection of the Deletion

PCR amplification of the 484-bp segment was performed on DNA from 75 patients with Gaucher disease including 47 individuals with type 1 Gaucher disease, 13 individuals with type 2 disease and 15 individuals with type 3 disease. Normal individuals and patients without the 55-bp deletion displayed a single 484-bp band by ethidium bromide staining (Fig. 2). Three patients, two with type 1 Gaucher disease and one with type 2 Gaucher disease, were heterozygous for a 55-bp deletion as demonstrated by the presence of a smaller 429-bp band (Fig. 2). For each of these three patients, the DNA amplified from each allele was sequenced individually. The sequencing confirmed the 55-bp deletion in exon 9.

Amplification Refractory Mutation System (ARMS)

The ARMS method [Mistry et al., 1992] was used to screen for mutation N370S in patients with and without the 55-bp deletion (Fig. 3). This technique amplifies a region which encompasses the 55-bp pseudogene gap. The PCR products, amplified from the glucocerebrosidase gene and pseudogene, respectively, are differentiated by a 55-bp size difference. If the larger PCR product amplified from the gene is absent, it is assumed the

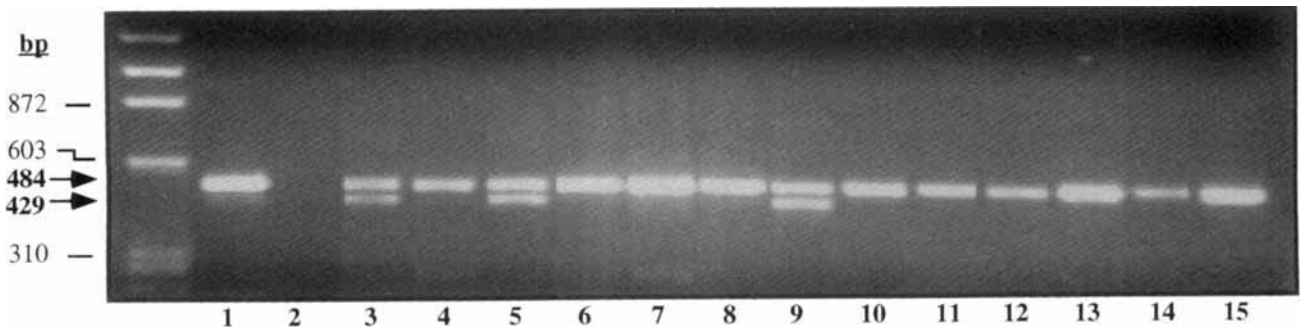

Fig. 2. Detection of the 55-bp deletion mutation. The 429-bp fragment (shown by the lower arrow) results from the 55-bp deletion. **Lane 1**: Amplification from a plasmid carrying the glucocerebrosidase gene. **Lane 2**: Amplification from a plasmid carrying the glucocerebrosidase pseudogene. **Lanes 3–15**: Amplified DNA from patients with type 1 (lanes 3–7), type 2 (lanes 8–11) and type 3 (lanes 12–15) Gaucher disease. The amplified DNA shown in lanes 3, 5, and 9 demonstrate heterozygosity for the deletion.

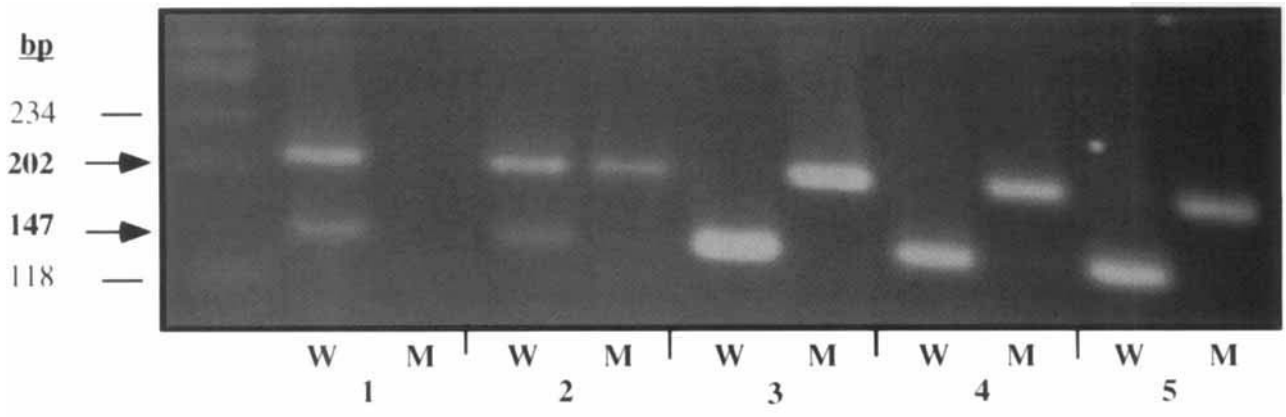

Fig. 3. Amplification refractory mutation system (ARMS) analysis for the N370S mutation. W, wild-type allele (primers 1 and 3); M, mutant allele (primers 2 and 3). Amplified DNA fragments from **lane 1**: A control individual. **Lane 2**: A patient heterozygous for N370S. **Lane 3**: A patient homozygous for N370S. **Lanes 4** and **5**: Patients with the N370S mutation on one allele and the 55-bp deletion on the second allele.

mutation N370S is present in both alleles. Thus, a patient with genotype N370S/del 55 (Fig. 3, patients 4 and 5) would not be distinguished from a N370S homozygote (Fig. 3, patient 3) using this method. Direct PCR sequencing of each allele confirmed the presence of the deletion in one allele and the presence of mutation N370S in the second allele.

DISCUSSION

Since certain mutant alleles, especially N370S are relatively common in particular populations of Gaucher patients, there has been great incentive to develop techniques that rapidly screen for specific mutations. Published techniques have included the ARMS [Mistry et al., 1992], a colorimetric assay [Zimran et al., 1990], restriction enzyme digestions [Sidransky et al., 1992], "mismatched PCR" [Beutler et al., 1990] and allele-specific oligonucleotide hybridization [Eyal et al., 1990]. However, the increasing awareness of the complexity of the glucocerebrosidase locus and the existence of deletions and recombinant alleles [Beutler and Grabowski, 1995; Horowitz and Zimran, 1994], illuminates the limitations of many of these screening techniques. For example, if the 55-bp deletion occurs in a Gaucher patient in combination with another common allele, certain frequently used screening techniques may fail to distinguish a heterozygote from a homozygote. When the ARMS technique is used, a patient with an N370S allele and the 55-bp deletion will be mistaken for a N370S homozygote. Additionally, any method for mutation identification or screening designed using primers originating from the 55-bp pseudogene gap [Theophilus et al., 1989; Choy et al., 1991; Sidransky et al., 1992; Eyal et al., 1990; Zimran et al., 1990; Beutler et al., 1990; Laubscher et al., 1994] will fail to amplify one allele if this deletion is present. Gaucher patients have also been described with larger deletions [Beutler and Gelbart, 1994] and here too, PCR analysis could lead to the erroneous conclusion that the patient is homozygous for the mutation detected on the second allele. PCR-based screening has other limitations, as it will only identify abnormalities between the 2 primers used and may fail to detect complex alleles, fusions, and recombinant alleles. The technique described here will identify whether this specific 55-bp deletion is present, except when a recombination or fusion event occurs upstream to exon 8. However, other techniques [Beutler and Grabowski, 1995; Horowitz and Zimran, 1994; Beutler and Gelbart, 1994] are necessary to establish whether the deletion is part of a complex allele or fusion gene, or if another or larger deleted segment is involved.

In this paper we describe a simple method to detect this 55 bp deletion in exon 9. This deletion was encountered in 4% of the 75 samples from patients with Gaucher disease studied. We recommend that any laboratory depending upon the ARMS method [Mistry et al., 1992] or using primers originating from this gap region [Theophilus et al., 1989; Choy et al., 1991; Sidransky et al., 1992; Eyal et al., 1990; Zimran et al., 1990; Beutler et al., 1990; Laubscher et al., 1994] initially determine whether this 55-bp segment is absent to enable more accurate genetic counseling based upon genotype.

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